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14. ABSTRACT The androgen receptor (AR) is a ligand-activated transcription factor that binds androgen response elements (ARE) in the promoters of target genes. When bound to ARES, the receptor recruits chromatin-remodeling complexes, components of the general transcription machinery, and coregulator proteins. Using the N-terminus of AR as bait in the repressed transactivator yeast two-hybrid system, TATA binding protein-associated factor 1 (TAF1) was identified. TAF1, a multifunctional protein that contains acetylation, ubiquitin activating and kinase domains, can interact with several proteins to promote or suppress gene transcription. We showed that AR and TAF1 co-immunoprecipitated in nuclear extracts from LNCaP cells, an AR-containing prostate cancer cell line. Furthermore, using ChIP assays with LNCaP cells, we found that both AR and TAF1 associated with an ARE in the proximal promoter of the PSA gene. To assess if TAF1 can modulate AR transcription, we performed transfection assays with androgen responsive luciferase reporters. Our results indicate that overexpression of TAF1 enhances AR activity several fold in LNCaP cells, whereas siRNA knockdown of TAF1 decreases AR transactivation. To differentiate between the direct effects of TAF1 on AR activity from general effects on transcription and to determine which TAF1 domains are involved in AR transactivation, we cloned and tested various functional domains of TAF1. Comparing AR-regulated and generic promoters, our results indicate that both the ubiquitin activating and the N-terminal kinase domains of TAF1 differentially enhance AR activity, but unlike full-length TAF1, have no effect on general gene transcriptional activity.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Hypothesis	4
Specific Aims	4
Progress and Results	4
TAF1 and androgen response element association on PSA gene	4
TAF1 can differentially enhance AR transcriptional activity via ubiquitin activating and N-terminal kinase domains	5
Reportable Outcomes	8
Conclusion	8
References	9

A. INTRODUCTION

Prostate cancer is the most commonly diagnosed non-skin cancer in men and one of the leading causes of cancer death. Androgen withdrawal therapies are still the most effective treatment for advanced disease, although it eventually progresses to the lethal androgen-independent stage. Evidence suggests that most cells in androgen-independent tumors retain androgen receptor (AR) that maintains its transcriptional activity through alternative pathways (1-7). Using the N-terminal domain of AR (NTD) as bait in the repressed transactivator yeast two-hybrid system, TATA binding protein associated factor 1 (TAF1) was detected (8). TAF1 is a multifunctional protein that contains acetylation (HAT), ubiquitin activating/conjugating (E1/E2) and bipartite, kinase domains consisting of N- and C-terminal kinases (NTK and CTK, respectively). TAF1 can interact with several proteins to promote or suppress gene transcription (9-12). However, its effect on AR and prostate cancer is not known. The focus of this study is to identify if and how TAF1 modulates AR transcription, and to determine the role of TAF1 in prostate cancer progression. In particular, this research proposal will test the hypothesis that TAF1 directly modulates AR activity, and aids in the development and maintenance of androgen-independent prostate cancer. We are testing this hypothesis with the following four specific aims:

- Specific Aim 1: To determine whether the TAF1/AR interaction specifically modulates AR transactivation.
- Specific Aim 2: To map functional interaction site(s) of AR and TAF1.
- Specific Aim 3: To determine if modulation of AR transactivation by TAF1 involves phosphorylation, acetylation, or ubiquitination of AR.
- Specific Aim 4: To assess the expression profile of TAF1 versus AR in prostate tumors:
 - a) To characterize TAF1 and AR expression at mRNA and protein levels in the *Pten*^{-/-} prostate-specific knock out mouse model.
 - b) To determine the expression level of TAF1 and AR in malignant versus benign human prostate tissues.

B. PROGRESS AND RESULTS

Specific Aim 1: To determine whether the TAF1/AR interaction specifically modulates AR transactivation.

A. TAF1 and AR are associated with the PSA promoter

We already showed that AR and TAF1 are co-immunoprecipitated in nuclear extracts from LNCaP cells, an AR-containing prostate cancer cell line (Figure 1) (unpublished data). Since TAF1 is a component of the transcriptional machinery, if TAF1 does associate with AR it is likely this interaction occurs on the promoter of an AR response gene. Chromatin immunoprecipitation (ChIP) assays were performed to determine if TAF1 and AR co-localize on prostate specific antigen (PSA) promoter. PSA is one of the most well characterized androgen-

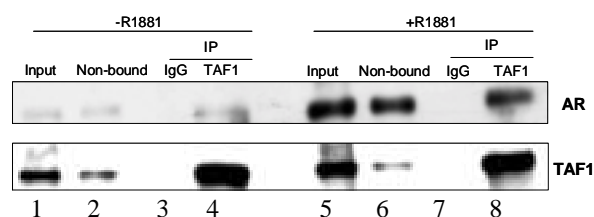


Figure 1: Androgen receptor interacts with TAF1 *in vivo*. LNCaP cells were transfected with pCS2+HA-TAF1 and grown in the absence (lanes 1-4) or presence (lanes 5-8) of 10 nM R1881 for 4 h prior to lysis. Nuclear extracts were incubated with an anti-TAF1 antibody (lanes 4 and 8) or normal mouse IgG control (lanes 3 and 7). Protein complexes were pulled-down using recombinant Protein A/G–agarose beads prior to SDS/PAGE and Western blot analysis, with antibodies against AR (top row) and TAF1 (bottom row). Lanes 1 and 5 inputs, lanes 2 and 6 non-bound proteins.

regulated genes. The PSA promoter contains several AREs and a distal androgen response region (13). ChIP assay was performed in the LNCaP cells with or without synthetic androgen (R1881). After cross linking, cells were lysed, sonicated, then immunoprecipitated with anti TAF1, AR or IgG. Cross links were reversed and the proximal PSA and actin promoters were specifically PCR amplified. The result showed that both TAF1 and AR associated with the PSA promoter. This association seemed to be specific since neither bound to actin promoter (Figure 2).

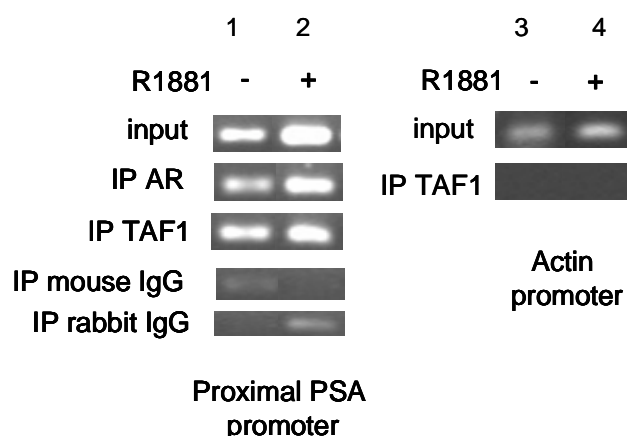


Figure 2: TAF1 and AR associate with an ARE in the proximal promoter of the PSA gene. After 3 days growing in charcoal stripped serum, LNCaP cells were treated either with 1nM R1881 or vehicle for 4 h followed by cross-linking of the proteins and DNA with formaldehyde. Cells were then lysed and sonicated to create a population of chromatin fragments ranging from 200 to 1,000 base-pairs. TAF1 was then immunoprecipitated along with AR (positive control) or normal IgG (negative control). The immunoprecipitated DNA was purified and analyzed by PCR using primers for the proximal promoter of PSA (lane 1 & 2) or actin promoter (lane 3 & 4, negative controls). PCR negative controls were also performed for the non-ARE sequences of the PSA promoter (data not shown).

TAF1 can differentially enhance AR transcriptional activity via ubiquitin activating and N-terminal kinase domains

To assess if TAF1 modulates AR transcription, transient transfection transactivation assays were performed using LNCaP cells. Cells were transiently co-transfected with increasing amounts of TAF1 or empty vector, a constant amount of an androgen responsive reporter vector (pPSA-

Luc or pARR3tk-Luc), and the Renilla luciferase vector (pRL-TK) as an internal control. Cells were then treated with or without R1881 for 24 h prior to harvesting for analysis. As can be seen in Figure 3, ligand-dependent AR activity was enhanced with increasing TAF1 expression more than three-fold with ARR3tk-Luc (3A) and two-fold with PSA-Luc (3B) vectors. Western blot was also performed to confirm the expression levels of HA-TAF1 and AR proteins (Figure 3).

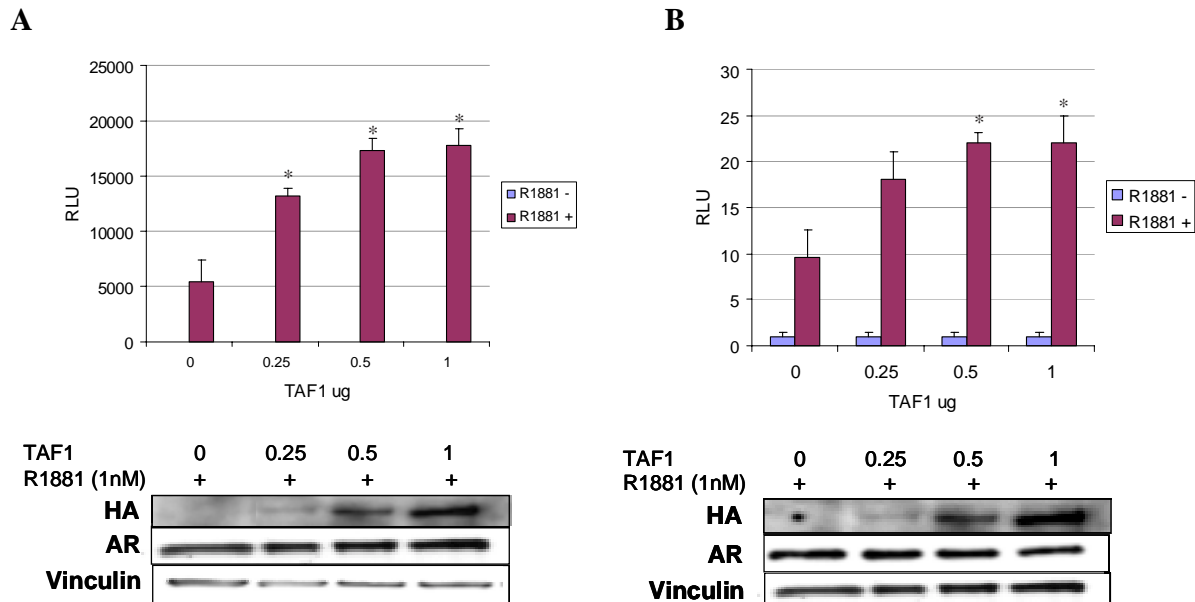


Figure 3: TAF1 enhances AR transcriptional activity in LNCaP cells. LNCaP cells were co-transfected with increasing amount of HA tagged full length TAF1 (pCS2+/TAF1), pRL-TK-Renilla (83 ng/well), and pARR3-tk-Luc (167 ng/well) (A) or pPSA-Luc (1ug/well) (B). Total DNA was kept constant at 1250 ng/well (A) or 2500 ng/well (B) with addition of pCS2+ vector. Transfected cells were growing in the presence or absence of R1881 for 24 h before harvesting for luciferase assay. Luciferase units (RLU) are expressed relative to protein values for each sample. All luciferase values are given as the mean (\pm SEM) of triplicate readings. Graphs are representative of the 3 independent experiments. * indicates a $p < 0.01$ compared to empty vector control.

To check the effect of TAF1 on AR activity in other prostate cancer cell line, similar transient transfection was carried on PC3 cells transfected with AR. Again in the absence of ligand, AR activity was negligible and did not change with TAF1 over-expression. However in the presence of R1881, over-expression of TAF1 enhances AR activity more than two-fold as shown in Figure 4. Since the absolute numbers of pRL-TK-Renilla, as a non androgenic reporter was also enhanced by TAF1 over-expression in both LNCaP and PC3 cells, the ARR3tk-Luc numbers were normalized with total protein values for each sample.

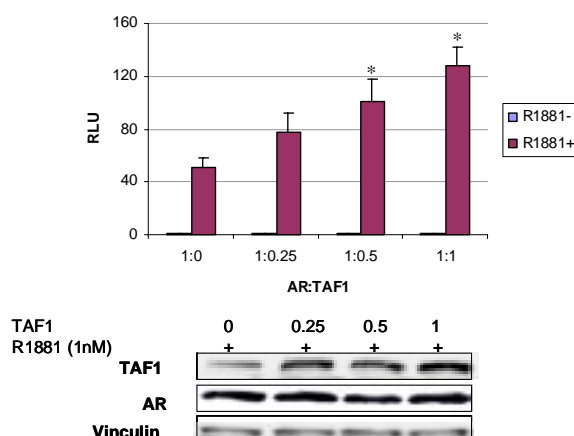


Figure 4: TAF1 enhances AR transcriptional activity in PC3 cells. PC3 cells were co-transfected with full length AR (250 ng/well) and increasing amount of full length TAF1, pARR3-tk-Luc, pRL-TK-Renilla, as described above. Total DNA was kept constant at 1500 ng/well with addition of pCS2+ vector. Transfected cells were growing in the presence or absence of R1881 for 24 h before harvesting for luciferase assay. RLU are expressed relative to protein values for each sample. All luciferase values are given as the mean (\pm SEM) of triplicate readings. Graphs are representative of the 3 independent experiments. * indicates a $p < 0.01$ compared to empty vector control.

To observe the effect of TAF1 knock-down on androgen and non androgen responsive reporters, siRNA for TAF1 or control siRNA were used in transient transfection assays (10) . As shown in the Western blot of Figure 5, protein level of TAF1 was decreased by increasing concentration of siRNA for TAF1, while vinculin and AR protein levels remained constant. Furthermore at 10nM siRNA, AR transcriptional activity was decreased about three-fold in both LNCaP and PC3 cells (Figure 5). Interestingly, the Renilla activity, a non-androgen responsive reporter was also decreased.

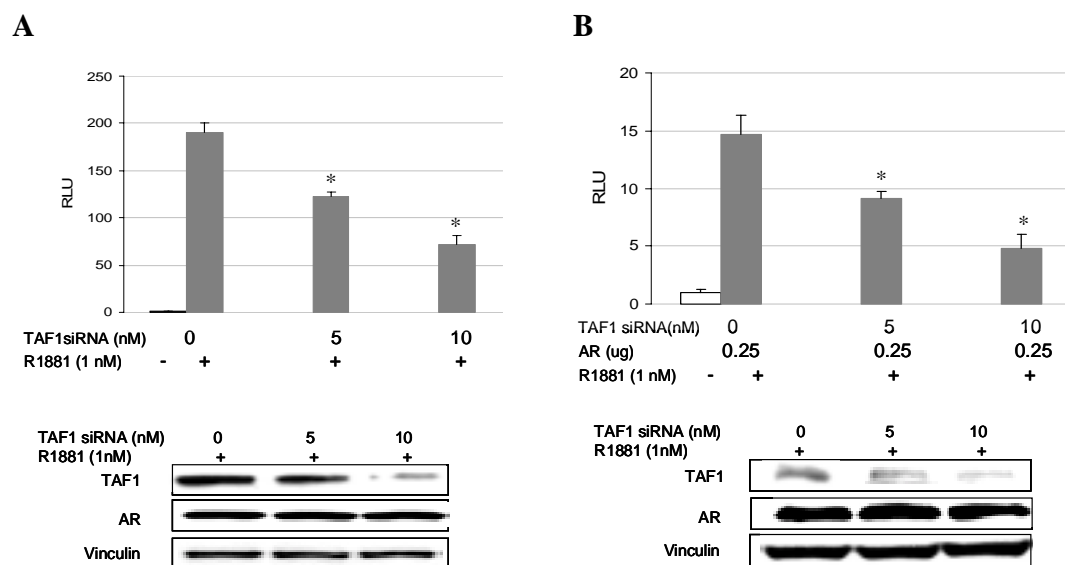


Figure 5: TAF1 knockdown decreases AR transcriptional activity in both LNCaP and PC3 cells. LNCaP (A) or PC3 transfected with AR (B) were co-transfected with increasing amount TAF1 siRNA, pARR3tk-Luc, and pRL-TK-Renilla as described before. Total siRNA was kept constant at 10 nM with addition of control siRNA. Transfected cells were growing for 24 h in charcoal stripped serum followed by another 24 h in the presence or absence of R1881 before harvesting for luciferase assay. RLU are expressed relative to protein values for each sample. All luciferase values are given as the mean (\pm SEM) of triplicate readings. Graphs are representative of the 3 independent experiments. * indicates a $p < 0.01$ compared to control siRNA.

Modulation of TAF1 levels also affected pRL-TK activity as expected, since TAF1 belongs to general transcription factor family. To differentiate between the direct effects of TAF1 on AR activity from general effects on transcription and to determine which TAF1 domains are involved in AR transactivation, we cloned and tested various functional domains of TAF1 as shown in Figure 6. The NTK and E1/E2 domains of TAF1 enhanced AR activity in LNCaP cells by two-fold and three-fold, respectively, while the HAT or CTK domains did not significantly modulate AR activity. Importantly unlike the full-length TAF1, individual domains had no effect on the pRL-TK reporter (data not shown), suggesting that the effect of NTK and E1/E2 domains of TAF1 are specific for AR.

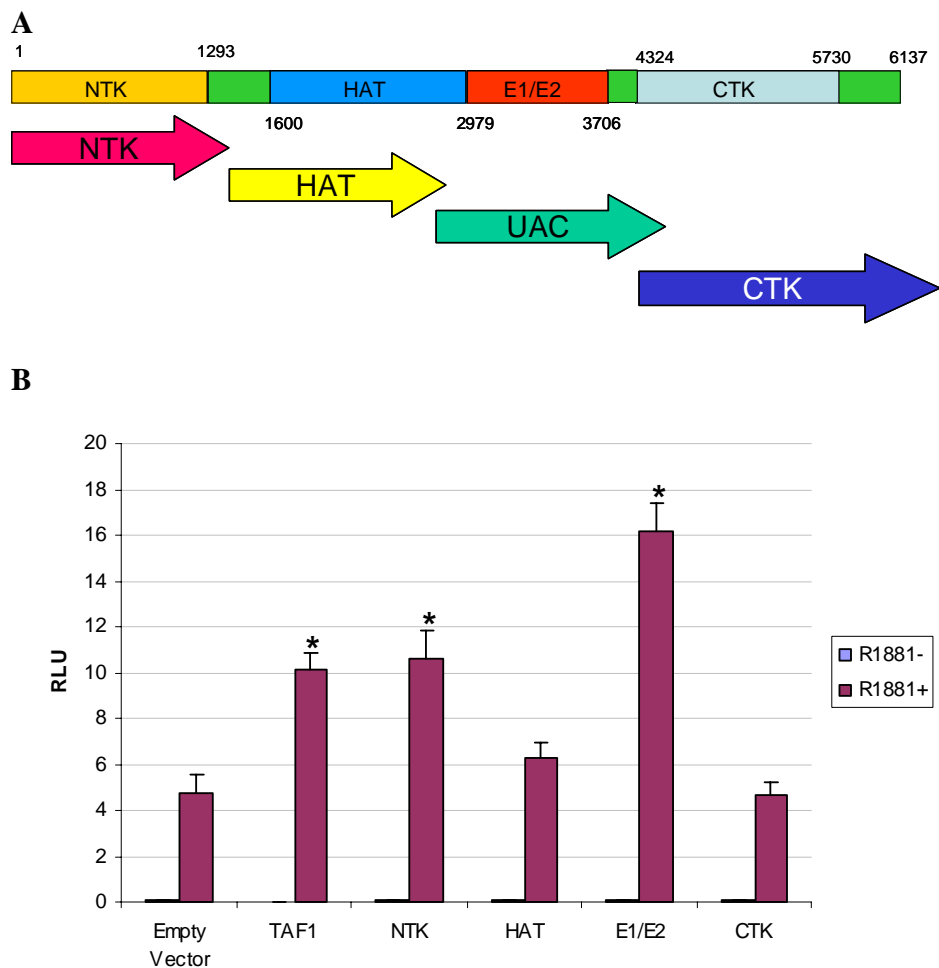


Figure 6: Full-length TAF1, TAF1-NTK, and E1/E2-TAF1 enhance AR transcriptional activity in a ligand dependent manner. A. Schematic presentation of TAF1 cDNA and its different domains that individually were cloned using the Topo cloning kit (invitrogen). B. LNCaP cells were co-transfected with full length TAF1 (pCS2+/TAF1) or one of its four domains (pcDNA3.1-V5/His-NTK, HAT, E1/E2, or CTK) (1 ug/well), pARR3-tk-Luc (167 ng/well) and pRL-TK-Renilla (83 ng/well). Transfected cells were growing in the presence or absence of R1881 for 24 h before harvesting for luciferase assay. Luciferase units (RLU) are expressed relative to protein values for each sample. All luciferase values are given as the mean (\pm SEM) of triplicate readings. Graphs are representative of the 3 independent experiments. * indicates a $p < 0.01$ compared to empty vector control.

C. REPORTABLE OUTCOME

A summary of the above data has been submitted for the Endocrine Society's 90th Annual Meeting in 2008.

D. CONCLUSION

Recent evidence suggests that AR-specific gene regulation may occur through interactions with unique coregulatory proteins. Since the N-terminus of AR (AR-NTD) is the least conserved,

protein interactions in this region may dictate receptor-specific coregulation capacity. To identify novel and specific AR-interacting proteins, the AR-NTD was used as bait in the repressed transactivator yeast two-hybrid system (RTA) (14) to screen a prostate carcinoma cell line (LNCaP) cDNA library. Using this system several clones that coded for AR-interacting proteins were detected including TAF1. By co-immunoprecipitation, we showed that TAF1 is associated with AR in the nucleus of LNCaP cells. Furthermore by ChIP assay, we found that this association is at the proximal promoter of the PSA gene. Using transient transfection reporter assays, we were also able to show that AR is differentially modulated through the E1/E2 and NTK domains of TAF1. Currently, we are mapping the TAF1/AR domains interaction (aim 2) and investigating the mechanism(s) by which TAF1 enhances AR transcriptional activity (aim 3).

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